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Efficacy of API 20C and ID 32C Systems for Identification of Common and Rare Clinical Yeast Isolates

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The abilities of the API 20C and ID 32C yeast identification systems to identify 123 common and 120 rare clinical yeast isolates were compared. API 20C facilitated correct identification of 97% common and 88% rare isolates while ID 32C facilitated correct identification of 92% common and 85% rare isolates.

The incidence of fungal infections has increased significantly over the past two decades. The National Nosocomial Infections Surveillance System (Atlanta, Ga.) has found a sharp rise in the incidence of yeast fungemias between 1980 and 1989 (1). Since many of the yeasts associated with human infections have been found to be innately resistant or to develop resistance to the most common antifungal agents, the introduction of appropriate therapy for these invasive infections depends largely on the rapid and accurate identification of the etiologic agents. For example, *Candida lusitaniae* has been reported to be resistant in vitro to amphotericin B, while *Candida krusei* and *Candida glabrata* have been found to be resistant to fluconazole (2, 3, 13, 15).

The classical methods used in the identification of yeast pathogens, such as the Wickerham and auxanographic techniques, are time consuming and technically complex (11). The increased incidence of yeast infections has stimulated the development of rapid and accurate manual and automated commercial systems for the identification of these pathogens. Ideally, these products should have the following features: (i) rapid and precise identification of yeasts isolated from all types of clinical specimens; (ii) ease of handling and inoculation to permit rapid processing of multiple isolates; and (iii) an ability to identify isolates that are less commonly recovered from specimens. The aim of this study was to compare the efficacy of the ID 32C system (bioMérieux, Marcy l'Etoile, France) commonly used in European countries to that of the API 20C yeast identification system (bioMérieux Vitek, Inc., Hazelwood, Mo.), one of the most commonly used yeast identification systems in the United States.

Yeasts. A total of 243 isolates of yeasts and yeast-like organisms comprising 123 commonly recovered isolates and 120 isolates of less frequently encountered taxa were used in these studies (see Tables 1 and 2). The yeasts included in these studies had been definitively identified by conventional biochemical and physiological methods (11). Yeasts and yeast-like organisms that were found in ≥1% of all yeast-positive clinical specimens were considered common clinical isolates (12a). The organisms were maintained on potato dextrose agar slants at −20°C in the culture collection of the Mycology Laboratories, Wadsworth Center, New York State Department of Health, Albany, N.Y. Additional clinical isolates came from bioMérieux Vitek, Inc., and were maintained in tryptic soy broth–10% glycerol at −70°C or in sterile distilled water at room temperature. Isolates to be studied were serially subcultured at least twice on 100-mm petri plates containing 25 ml of Sabouraud glucose agar at 30°C for 18 to 24 h prior to the inoculation of the two identification systems.

API 20C. All yeast identification procedures were conducted in accordance with the manufacturer's instructions. Portions of growth of each isolate were aseptically transferred from a freshly inoculated stock culture to an ampule of API 20C basal medium and then emulsified to give a density of 1+ on a Wickerham card. Each well of the API 20C strip was inoculated with the suspension, and the strip was placed in the incubation tray provided by the manufacturer, covered loosely with a lid, and incubated at 30°C for 72 h. Reactions were visually examined at 72 h and determined to be positive or negative based on the presence or absence of turbidity in the carbohydrate wells. A seven-digit biocode was generated on the basis of these observations by assigning a weighted score to positive reactions. These codes were then compared to those listed in the API 20C Analytical Profile Index. Identifications listed in the index as excellent, very good, or acceptable were accepted as correct, and no supplemental tests were conducted. Additionally, presumptive good likelihood (low selectivity) identifications that required microscopic morphology for confirmation were considered correct without the need of supplemental testing. In contrast, supplemental tests, e.g., KNO_3 utilization, growth at 42°C, and urease production, were used to confirm remaining presumptive or questionable identifications.

ID 32C. This system consists of a single-use disposable plastic strip with 32 wells containing substrates for 29 assimilation tests (carbohydrates, organic acids, and amino acids), one susceptibility test (cycloheximide), one colorimetric test (esculin), and a negative control. The yeast identification procedures were conducted in accordance with the manufacturer's instructions. A portion of growth from well-isolated colonies of each isolate was aseptically transferred from a freshly inoculated stock culture to sterile distilled water to prepare a suspension with a final turbidity equivalent to McFarland standard #2. Five drops of this suspension was then dispensed to an ampule of C medium provided by the manufacturer and homogenized to prepare an even dispersion of inoculum. After homogenizing, the inoculum suspension was used to inoculate the wells in the strip, the lid of the strip was replaced, and the system was incubated at 30°C for 48 h. The strips were then visually examined, and growth was determined to be positive or negative

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TABLE 1. Identification of common clinical yeast isolates with the API 20C and ID 32C systems

Organism	No. of isolates	No. of isolates tested with API 20C that were:				No. of isolates tested with ID 32C that were:			
		Correctly identified	Correctly identified by additional testing	Not identified	Misidentified	Correctly identified	Correctly identified by additional testing	Not identified	Misidentified
Candida albicans	10	9		1		8		2	
Candida glabrata	8	8				8			
Candida guilliermondii	12	11		1		10		2	
Candida krusei	10	10				10			
Candida parapsilosis	12	11		1		12			
Candida tropicalis (sucrose positive)	8	8				8			
Cryptococcus albidus	8	8				8			
Cryptococcus neoformans	18	18				14		4	
Geotrichum sp.	10	10				10			
Rhodotorula sp.	11	6	5			3	7	1	
Saccharomyces cerevisiae	9	9				8		1	
Trichosporon beigelii	7	6			1	7			
Total	123	114 (93%)	5	3	1	106 (86%)	7	10	0

based upon the presence or absence of turbidity in the wells. The results were transformed into numerical biocodes, and the isolates were identified through the use of the ID 32C Analytical Profile Index.

Quality control. Each system was tested with the manufacturer's recommended quality control test organisms before starting the formal evaluation. These included *Cryptococcus laurentii* (ATCC 18803) and *Blastoschizomyces capitatus* (ATCC 10663) for the API 20C system and *Candida guilliermondii* (ATCC 6260) for the ID 32C system.

API 20C results. Correct identifications were obtained for 114 (93%) of the common yeast isolates with the API 20C without using supplementary tests. Of the remaining isolates, five (4%) were appropriately identified with additional tests (Table 1). In contrast, 105 (88%) of the rarely encountered isolates were identified correctly with no supplemental tests, and an additional 2 (2%) isolates were correctly identified when the manufacturer's recommended supplemental tests were used in conjunction with the assimilation profiles (Table 2). The biocodes of several rarely recovered isolates (e.g., *Candida sake*,

TABLE 2. Identification of rare clinical yeast isolates with the API 20C and ID 32C systems

Organism	No. of isolates	No. of isolates tested with API 20C that were:				No. of isolates tested with ID 32C that were:			
		Correctly identified	Correctly identified by additional testing	Not identified	Misidentified	Correctly identified	Correctly identified by additional testing	Not identified	Misidentified
Blastoschizomyces captitatus	6	6				6			
Candida ciferrii	7	6		1		5		2	
Candida famata	7	7				6		1	
Candida kefyr	5	5				5			
Candida lambica	9	8			1	8			1
Candida lipolytica	10	10				10			
Candida lusitaniae	7	7				7			
Candida norvegensis	2		2			2			
Candida tropicalis (sucrose negative)	2	2						1	1
Candida rugosa	3	3				3			
Candida sake	5			5		5			
Candida zeylanoides	5	5				5			
Cryptococcus humicolus	6	5		1		4		2	
Cryptococcus laurentii	7	7				6		1	
Cryptococcus terreus	6	6				6			
Cryptococcus uniguttulatus	4	4				4			
Hansenula anomala[a]	7	7				6		1	
Pichia farinosa[a,b]	5	5				4		1	
Pichia membranaefaciens[a,b]	4			4		3		1	
Pichia ohmeri[a,b]	1	1				1			
Prototheca zopfii	3	3						3	
Prototheca wickerhamii	2	2						2	
Torulaspora rosei[a]	6	6				6			
Zygosaccharomyces spp.	1			1		1			
Total	120	105 (88%)	2	12	1	102 (85%)	0	15	2

[a] Identified as the anamorphic form.
[b] The biocode for this organism was derived from Profiles for Unusual Fungal Isolates in the Clinical Laboratory.

TABLE 3. Summary of identification of yeast isolates with the API 20C and ID 32C systems

Type of isolates	No. of isolates	No. of isolates tested with API 20C that were:				No. of isolates tested with ID 32C that were:			
		Correctly identified	Correctly identified by additional testing	Not identified	Misidentified	Correctly identified	Correctly identified by additional testing	Not identified	Misidentified
Common clinical	123	114	5	3	1	106	7	10	
Rare clinical	120	105	2	12	1	102		15	2
Total	243	219 (90%)	7	15	2	208 (86%)	7	25	2

Pichia membranaefaciens, and *Zygosaccharomyces* species) generated assimilation patterns in the API 20C which were not listed in its Analytical Profile Index and were recorded as no identification.

ID 32C results. Appropriate identifications were obtained with the ID 32C system for 106 (86%) of the frequently recovered yeasts without supplemental tests. Of the remaining isolates, 7 (6%) were appropriately identified by the recommended supplemental tests, and 10 were not identified (Table 1). Of the 120 less common yeasts tested, 102 (85%) were identified with the ID 32C while 15 were not identified. With all 243 yeasts employed in this study, the ID 32C identified 88% of the isolates correctly with supplemental testing.

These studies demonstrate that the ID 32C is as efficacious as the commonly used API 20C in the identification of yeasts and yeast-like pathogens which are isolated in the clinical laboratory (Table 3).

This is, to the authors' knowledge, the first study to compare the API 20C, one of the most common yeast identification systems in the United States, with the ID 32C, the kit more commonly used in Europe. Our results with API 20C are compatible with those of other published reports (4, 6, 7, 10, 12, 14). For example, Fenn et al. (7) reported that 99% of the yeast isolates were identified correctly with API 20C, while Davey et al. (5) found 88% appropriate identifications with the API 20C. We found that all of the common yeast isolates were correctly identified with the API 20C when supplemental tests were employed. In the case of the ID 32C, we observed that 98% of all yeast isolates could be identified when the biocodes were combined with the results of supplemental tests. This compares favorably with the 92% identification rate reported by Fricker-Hidalgo and coworkers (9). While both products are effective in the identification of relatively common yeasts, their application is somewhat more limited in the accurate identification of less frequently recovered taxa. These limitations are probably attributable, in part, to the databases currently employed in the profile indexes.

Davey and coworkers (5) found that isolates of *C. glabrata* did not assimilate trehalose within the API 20C's 72-h incubation time frame and as a result, could not be identified with this system. In contrast, we noted that the API 20C provided for the accurate identification of all isolates of this yeast without the need for supplemental identification tests. Fricker-Hidalgo et al. (8) noted that the presence of additional substrates in the ID 32C allowed them to identify unknown isolates without morphologic information on the yeasts. However, we found that 25 yeast isolates included in this study could not be identified by either system.

We found that the two identification systems were comparable in their overall efficacy, but results could be obtained 24 h earlier with the ID 32C system. However, the interpretation of test results obtained with the ID 32C system was more difficult and required greater experience than did interpretation of those obtained with the API 20C. The presence of 12 more substrates in the ID 32C should have the potential for the identification of a more diverse set of clinically important yeasts, but our results did not indicate any superiority of ID 32C over API 20C. It is possible that geographic origin may have played a role in the higher number of isolates that could not be identified with ID 32C, since the ID 32C database was developed with European isolates, whereas the API 20C database was developed with U.S. isolates. To test the geographic origin hypothesis, one would have to perform the study with the same isolates in both regions.

REFERENCES

1. **Banerjee, S. N., T. G. Emori, D. H. Culver, R. P. Gaynes, W. R. Jarvis, T. Horan, J. R. Edwards, J. Tolson, T. Henderson, W. J. Martone, and the National Nosocomial Infections Surveillance System.** 1991. Secular trends in nosocomial primary bloodstream infections in the United States, 1980–1989. Am. J. Med. **91**(Suppl. 3B)**:**86S–89S.
2. **Blinkhorn, R. J., D. Adelstein, and P. J. Spagnuolo.** 1989. Emergence of a new opportunistic pathogen, *Candida lusitaniae*. J. Clin. Microbiol. **27:**236–240.
3. **Bodey, G. P.** 1992. Azole antifungal agents. Clin. Infect. Dis. **14**(Suppl. 1)**:** S161–S169.
4. **Buesching, W. J., K. Kurek, and G. D. Roberts.** 1979. Evaluation of the modified API 20C system for identification of clinically important yeasts. J. Clin. Microbiol. **9:**565–569.
5. **Davey, K. G., P. M. Chant, C. S. Downer, C. K. Campbell, and D. W. Warnock.** 1995. Evaluation of the Auxacolor System, a new method of clinical yeast identification. J. Clin. Pathol. **48:**807–809.
6. **El-Zaatari, M., L. Pasarell, M. R. McGinnis, J. Buckner, G. A. Land, and I. F. Salkin.** 1990. Evaluation of the updated Vitek yeast identification data base. J. Clin. Microbiol. **28:**1938–1941.
7. **Fenn, J. P., H. Segal, B. Barland, D. Denton, J. Whisenant, H. Chun, K. Christofferson, L. Hamilton, and K. Carroll.** 1994. Comparison of updated Vitek yeast biochemical card and API 20C yeast identification systems. J. Clin. Microbiol. **32:**1184–1187.
8. **Fricker-Hidalgo, H., B. Lebeau, P. Kervroedan, O. Faure, P. Ambroise-Thomas, and R. Grillot.** 1995. Auxacolor, a new commercial system for yeast identification: evaluation of 182 strains comparatively with ID 32C. Ann. Biol. Clin. **53:**221–225.
9. **Fricker-Hidalgo, H., O. Vandapel, M.-A. Duchesne, M.-A. Mazoyer, D. Monget, B. Lardy, B. Lebeau, J. Freney, P. Ambroise-Thomas, and R. Grillot.** 1996. Comparison of the new API Candida system to the ID 32C system for identification of clinically important yeast species. J. Clin. Microbiol. **34:** 1846–1848.
10. **Land, G. A., B. A. Harrison, K. L. Hulme, B. H. Cooper, and J. C. Byrd.** 1979. Evaluation of the new API 20C strip for yeast identification against a conventional method. J. Clin. Microbiol. **10:**357–364.
11. **Lin, C. C. S., and D. Y. C. Fang.** 1987. Conventional and rapid methods for yeast identification. Crit. Rev. Microbiol. **14:**273–288.
12. **Salkin, I. F., G. A. Land, N. J. Hurd, P. R. Goldson, and M. R. McGinnis.** 1987. Evaluation of YeastIdent and Uni-Yeast-Tek yeast identification systems. J. Clin. Microbiol. **25:**624–627.

12a. **Salkin, I. F., and G. D. Roberts.** Unpublished data.

13. **Stein, D. K., and A. M. Sugar.** 1989. Fungal infections in the immunocompromised host. Diagn. Microbiol. Infect. Dis. **12:**221S–228S.
14. **St.-Germain, G., and D. Beauchesne.** 1991. Evaluation of the MicroScan rapid yeast identification panel. J. Clin. Microbiol. **29:**2296–2299.
15. **Wingard, J. R., W. G. Merz, M. G. Rinaldi, T. R. Johnson, J. E. Karp, and R. Saral.** 1991. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N. Eng. J. Med. **325:**1274–1277.